

Februarg 5, 1885.

THE TREASURER in the Chair.

The Presents received were laid on the table, and thanks ordered for them.

The following Paper was read:—

- I. "The Relation of Bacteria to Asiatic Cholera." By E. KLEIN, M.D., F.R.S., Joint Lecturer on General Anatomy and Physiology at the Medical School of St. Bartholomew's Hospital, London. Received January 28, 1885.

I propose to bring before the Royal Society the results of an inquiry into the etiology of Asiatic cholera, undertaken, at the instance and expense of the Secretary of State for India, by myself, Dr. Gibbes, and Mr. Alfred Lingard while in India. This investigation will be published *in extenso* by the India Office, but permission has been granted to us to bring to the notice of the Society some of the more important points of our inquiry, particularly those regarding the relation of bacteria to Asiatic cholera. I shall supplement them by giving the results of further observations which I have made since my return from India.

As is now well known, Dr. Robert Koch, in an extensive inquiry into the etiology of cholera in Egypt, Calcutta, and in France, 1883-84, undertaken by him, Drs. Gaffky and Fisher, at the instance of the German Government, has arrived at certain conclusions, which briefly stated are these:

1. In all persons suffering from Asiatic cholera there occur in the rice-water stools during the acute stage of the disease certain well-characterised bacteria, which, on account of their curved shape, Koch called "comma bacilli."

2. These comma bacilli are mobile rods, of small size, of about the same thickness as tubercle bacilli, but only of half their length; they are always more or less curved, sometimes as much as to form half a circle; they vary in length according to the state of growth; they occur either singly or in couples, in the latter case arranged like an S.

3. The comma bacilli occur in great numbers in the mucus flakes as well as in the fluid of the choleraic evacuations. They occur in the lower part of the ileum of persons dead in the acute stage almost to

the exclusion of other bacteria, and in such great numbers that the lower part of the ileum may be considered to contain almost "a pure cultivation of comma bacilli."

4. The mucous membrane of the ileum, particularly that of the lower part, around and in the lymphatic glands located here—the solitary and Peyer's lymph-glands—exhibits in typical and rapidly fatal cases characteristic alterations: loosening and detachment of the epithelium of the surface and of that lining the glands of Lieberkühn; swelling of the mucous membrane and congestion of its blood-vessels, particularly at the peripheral portions of the lymph glands. These alterations are due to the presence, growth, and multiplication of the comma bacilli in these tissues, and the disease cholera is caused by the production on the part of these comma bacilli, and by the absorption on the part of the system of a special chemical ferment.

This state of the presence of the comma bacilli in the tissue is best pronounced in the lower part of ileum; higher up it is more limited, and gradually diminishes, and finally disappears in the upper part of the small intestine.

5. The blood and other tissues are free of any organisms.

6. The comma bacilli grow well outside the body at the ordinary temperature of the room, but better still at higher temperatures up to 38° or 40° C. They divide transversely; after division the two offsprings may remain joined end to end with shape of an S, and by further division they may grow into a spiral-like or wavy form. They grow well in the mucus flakes taken from the intestine, and placed on linen kept in a moist cell; they grow well on potato, in broth, in Agar-Agar jelly, in solid nourishing gelatine mixture (gelatine, peptone, and beef extract). In this latter substance they exhibit a peculiar and definite mode of growth not seen by Koch on any other bacteria. The comma bacilli require for their growth an alkaline medium; they are killed by acid, by drying, and various antiseptic media.

7. On account of their constant occurrence in the intestines of patients suffering from Asiatic cholera, on account of their absence in all other diseases of the intestine, and on account of their peculiar mode of growth in nourishing gelatine, Koch vindicates for these comma bacilli not only an important diagnostic value, but also considers them as the true cause of cholera.

8. Since his return to Germany, Koch has convinced himself of the correctness of the observations of Nicati and Rietsch, who maintain that cholera can be produced in dogs and guinea-pigs by injecting directly into the small intestine of these animals the comma bacilli taken either directly from the choleraic evacuations, or from artificial cultivations.

Our investigations enable us to say this :

1. Koch's statement as to the almost constant occurrence of comma bacilli in the rice-water stools of cholera patients is correct; the comma bacilli vary greatly in numbers in different stools and in different cases, in some being exceedingly scarce, in others numerous.

2. These comma bacilli vary greatly in length, some being twice and three times as long as others, some well curved as much as to form half a circle, others showing only just a slight bend. The name comma bacillus is inappropriate, as in reality they are vibrios.

3. The comma bacilli occur in the mucus flakes of the rice-water stools as well as in those taken from the ileum of a person dead of cholera. The sooner after death the examination is made, the fewer comma bacilli are found in the mucus flakes; in several typical rapidly fatal cases the mucus flakes taken from the ileum and examined soon after death (from between fourteen minutes and an hour or an hour and a half) contained the comma bacilli only very sparingly indeed, and not to the exclusion of other bacteria. Our investigations do not bear out Koch's statement as to the lower part of the ileum being in acute typical cases of cholera almost "a pure cultivation of comma bacilli." In not one of the many post-mortem examinations of typical acute cases have we found such a state.

4. The mucous membrane of the ileum of typical rapidly fatal cases, if examined soon after death, does not contain in any part any trace of a comma bacillus or any other bacteria, not even in the superficial loosened epithelium.

If the post-mortem examination is sufficiently delayed, comma bacilli and other bacteria may be found penetrating into the spaces of the mucous membrane.

The theory of Koch's as to the comma bacilli present in the mucous membrane secreting a chemical poison inducing the disease cannot, therefore, be correct.

5. Neither the blood nor any other tissue contains comma bacilli or any other micro-organisms of known character.

6. The behaviour of the comma bacilli in artificial media is not such as to justify their being considered as specific. They grow well in alkaline and neutral media, are not killed by acids, and their mode of growth in gelatine mixtures, however peculiar, is not more peculiar than that of other putrefactive bacteria; they show marked differences when grown in different media, but not more so than the ordinary putrefactive bacteria when compared in their growth with one another. The manner in which the choleraic comma bacilli grow in gelatine is identical with that shown by the comma bacilli of the mouth of healthy persons (Lewis) in that same medium.

7. Koch overlooked that "comma bacilli" occur in other intestinal

diseases, in the mouths of healthy persons, and as shown recently, even in some common articles of food.

8. The experiments performed by Koch and others on animals do not in the least prove that the comma bacilli are capable of producing cholera or any other disease. The results obtained by them are much easier explained in a manner opposed to that given by Koch and others.

9. There is direct evidence to show that the water contaminated with choleraic evacuations, and containing, of course, the comma bacilli, when used for domestic purposes, including drinking, by a large number of persons, did not produce cholera.

10. The mucus flakes taken from the small intestine of a typical rapidly fatal case of cholera contain numerous mucus corpuscles filled with peculiar minute straight bacilli; in this state they are found when the examination is made very soon after death; soon, however, the mucus corpuscles swell up and disintegrate, and then their bacilli become free.

The small bacilli are never missed in the mucus flakes. They are only one-third or one-fourth the length of the comma bacilli, and about half their thickness. They are non-mobile; they grow well in Agar-Agar jelly, but show in their mode of growth no peculiarity by which they could be considered as specific. When grown on the free surface of the nourishing material they form spores.

11. These small bacilli are not present in the blood, in the mucous membrane of the intestine, or in any other tissue.

12. Experiments made with these small bacilli on animals produced no result.

13. Since my return to London I have ascertained that the comma bacilli of cholera show two distinct modes of division, one the known one of transverse division, and a second one of division in length. When growing in Agar-Agar jelly at the ordinary temperature of the room, after some days the bacilli swell up owing to the appearance in their protoplasm of one or more vacuoles; as these vacuoles increase, so the comma bacilli become gradually changed first into plano-convex, then into oblong bi-convex, and ultimately into circular corpuscles. The longer the original comma bacillus, the larger the final circle. These circular organisms are mobile just as the comma bacilli, and by disintegration of the protoplasm at two opposite points two perfect more or less semicircular comma bacilli are formed. Growing the comma bacilli in Agar-Agar jelly kept at higher temperatures (30–40° C.), they multiply by transverse division only, but transferring these to Agar-Agar jelly and keeping this at the ordinary temperature of the room, they again gradually change into circular organisms, which, by division in the diameter of the circle, form two new comma bacilli.